



Increased Circulating Levels of Betatrophin in Newly Diagnosed Type 2 Diabetic Patients

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OBJECTIVE

Betatrophin, a newly identified hormone, has been recently characterized as a potent stimulator that increases the production and expansion of insulin-secreting β -cells in mice, but the physiological role of betatrophin remains poorly understood. This study measured for the first time serum betatrophin levels in newly diagnosed patients with type 2 diabetes (T2DM) and explored the correlations between its serum levels and various metabolic parameters in T2DM.

RESEARCH DESIGN AND METHODS

We analyzed the concentrations of betatrophin by ELISA in blood samples of 166 well-characterized individuals in whom anthropometric parameters, oral glucose tolerance test (OGTT), glycosylated hemoglobin, blood lipids, insulin sensitivity (1/homeostasis model assessment of insulin resistance [1/HOMA-IR] and Matsuda index [ISI_M]), and insulin secretion were measured. The participants were divided into newly diagnosed T2DM patients ($n = 83$) and age-, sex- and BMI-matched healthy control subjects ($n = 83$).

RESULTS

Serum betatrophin levels were significantly higher in T2DM patients than in healthy control subjects (613.08 [422.19–813.08] vs. 296.57 [196.53–509.46] pg/mL; $P < 0.01$). Serum betatrophin positively correlated with age, 2-h post-OGTT glucose (2hPG), and postprandial serum insulin (PSI), but negatively with 1/HOMA-IR and ISI_M in T2DM patients. In the control group, betatrophin was only positively associated with age. In T2DM subjects, multivariate regression analyses showed that age, 2hPG, and PSI were independent factors influencing serum betatrophin levels.

CONCLUSIONS

Circulating concentrations of betatrophin are significantly increased in T2DM patients. Our results suggest that betatrophin may play a role in the pathogenesis of T2DM.

Precise regulation of β -cell function is crucial for maintaining blood glucose homeostasis (1). In type 2 diabetes (T2DM), ambient insulin resistance forces β -cells to produce more insulin, which ultimately results in exhaustion of insulin production secondary to deterioration of β -cell functions. Unfortunately, neither pharmacotherapy nor insulin injections can reverse ongoing failure of β -cell function to prevent uncontrolled hyperglycemia and the devastating microvascular, neurologic, and macrovascular complications of diabetes. Treatments that replenish β -cell

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mass in diabetic patients could allow for the long-term restoration of normal glycemic control and thus represent a potentially curative therapy (2).

It has long been known that insulin resistance induces compensatory increases in β -cell mass and function to maintain normoglycemia, likely due to circulating growth factor(s) (3). The identification and characterization of these factors are of paramount importance, not only for a better understanding of endocrine pancreatic physiology and pathology, but also for the development of potential therapeutic approaches that their correct exploitation may offer. Gut-derived incretins such as glucagon-like peptide-1, adipocyte-derived adipokines including leptin and adiponectin, muscle-derived myokines such as interleukin-6, and thyroid-derived T3/T4 hormones have all been shown to increase β -cell mass, but the therapeutic manipulation of these hormones has been limited by their lack of specificity and modest effects (4). Work by the Melton laboratory (2) recently reported on betatrophin, a secreted protein of 198 amino acids that specifically increases β -cell mass in mice and therefore raises hope for regenerative β -cell therapy in humans.

Although betatrophin exerts strong antidiabetic properties in animal models, an exact knowledge of its bioactivity and its mode of action remains to be investigated. To explore the clinical relevance of betatrophin in humans, we measured its serum concentrations in normal subjects and newly diagnosed T2DM patients and analyzed its association with anthropometric and metabolic parameters.

RESEARCH DESIGN AND METHODS

Study Population

A total of 166 subjects were recruited in this study from October 2012 to November 2013: 83 healthy control subjects and 83 newly diagnosed T2DM patients. Oral glucose tolerance test (OGTT) was performed in all of the included participants. The diagnosis of T2DM was based on the American Diabetes Association diagnostic criteria 2011 (5). Subjects with impaired fasting glucose and/or impaired glucose tolerance were excluded. T2DM patients who were treated with oral hypoglycemic, hypolipidemic, and/or antihypertensive

agents were also excluded to avoid the possible confounding effects of medications. None of the control subjects was taking medications known to affect glucose tolerance and lipid metabolism. Individuals with type 1 diabetes, gestational diabetes, active hepatitis/liver cirrhosis, chronic renal failure on hemodialysis, congestive heart failure, or other known major disease were excluded from the study. Each subject was asked details of smoking status and alcohol consumption. All subjects enrolled in the study gave informed consent. The study protocol was in agreement with the guidelines of the ethics committee at our hospital.

Anthropometric and Biochemical Measurements

Weight (without shoes and in light outdoor clothing) and height were measured, and BMI (kg/m^2) was calculated by dividing weight (kilograms) by height squared (meters squared). Waist circumferences were measured at the narrowest point between the lowest rib and the uppermost lateral border of the right iliac crest, the hips were measured at their widest point, and the waist-to-hip ratio (WHR) was calculated. Seated blood pressure was taken by a trained nurse after the subjects had rested for 10 min. Blood samples were withdrawn from an antecubital vein after 10 h overnight fasting at the time of OGTT. After clotting, blood specimens were separated by centrifugation for 15 min at 1,000g. Serum samples were subsequently stored in aliquots without preservatives at -80°C for an average of 3 months until immediately before analysis of betatrophin. Plasma glucose levels were determined using the glucose oxidase method; insulin levels were measured using chemiluminescence; serum total cholesterol (TC), triglycerides, LDL cholesterol, and HDL cholesterol were measured using enzymatic methods; and alanine aminotransferase (ALT), aspartate aminotransferase (AST), and γ -glutamyl transpeptidase (GGT) were measured by kinetic method (Beckman Coulter Inc., Brea, CA). Glycosylated hemoglobin (HbA_{1c}) was measured by high-performance liquid chromatography (Arkray Inc., Kyoto, Japan).

Serum betatrophin levels were determined using a commercially available human ELISA kit (catalog number E11644h; Wuhan Eiaab Science, Wuhan, China)

with an intra-assay coefficient of variation (CV) of $\leq 4.8\%$ and an interassay CV of $\leq 7.2\%$. Human betatrophin ELISA was performed in duplicate according to the manufacturer's protocol. If duplicates had $>15\%$ CV, the sample was repeated. A calibration curve was constructed by plotting the absorbance values at 450 nm versus the betatrophin concentrations of the calibrators, and concentrations of samples were determined using this calibration curve. The lower and upper limits of detection of the ELISA were 78.0 and 5,000 pg/mL.

Calculations

Homeostasis model assessment of insulin resistance (HOMA-IR) and HOMA of insulin secretion (HOMA- β) was calculated using the 2004 computer program (6). The HOMA-IR primarily reflects hepatic insulin resistance, and its reciprocal ($1/\text{HOMA-IR}$) provides a measure of hepatic insulin sensitivity (7,8). The Matsuda index (ISI_M) incorporated both hepatic and muscle components of insulin resistance and correlated well with a euglycemic insulin clamp, as a measure of whole-body insulin sensitivity (9), and was calculated according to the published formula (10). Early-phase insulin release ($\text{InsAUC}_{30}/\text{GluAUC}_{30}$) was calculated as the total insulin area under the curve (AUC) divided by the total glucose AUC during the first 30 min of an OGTT (11). Total insulin release ($\text{InsAUC}_{120}/\text{GluAUC}_{120}$) was calculated using the ratio of total insulin AUC and total glucose AUC during 0–120 min of the OGTT (11–14).

Statistical Methods

All statistical analyses were performed using SPSS version 16.0 (SPSS Inc., Chicago, IL). Data are presented as mean \pm SD or median (25th and 75th percentiles) for continuous variables or as percentage for categorical variables. Betatrophin, ALT, AST, and GGT values were log transformed due to their nonnormal distribution. Independent Student *t* test was used to compare differences between case and control subjects. Categorical variables were examined by χ^2 test. Relationships among betatrophin, liver enzymes, blood glucose, lipids, insulin sensitivity, and insulin secretion were examined by calculation of partial correlation coefficients. Multivariate regression models were fit for betatrophin as a dependent variable, including all variables

of interest at the same time as independent variables to demonstrate the relative contribution of each of these variables to the outcome ones. The following independent variables were considered for the model: age, fasting plasma glucose (FPG), 2 h post-OGTT glucose (2hPG), HbA_{1c}, postprandial serum insulin (PSI), 1/HOMA-IR, and ISI_M. Only the variables that had a *P* value <0.05 were considered in the final fitted model. A two-sided value of *P* < 0.05 was considered statistically significant.

RESULTS

Characteristics of Study Participants

The clinical baseline characteristics of the subgroups studied (control and T2DM) are summarized in Table 1. Because subjects were matched for age and sex distribution, both parameters were similar between the two groups. In addition, there were no statistically significant differences between T2DM patients and control subjects with respect to BMI, WHR, blood pressure, smoking status, alcohol consumption, ALT, and AST. Compared with the control group, GGT, FPG, 2hPG, HbA_{1c}, TC, triglyceride, LDL cholesterol, fasting serum insulin (FINS), and PSI in T2DM group were significantly increased (*P* < 0.01 or *P* < 0.05), while HDL cholesterol, 1/HOMA-IR, ISI_M, HOMA-β, InsAUC₃₀/GluAUC₃₀, and InsAUC₁₂₀/GluAUC₁₂₀ were significantly decreased (*P* < 0.01 or *P* < 0.05). Serum betatrophin levels were higher in patients with T2DM than in control subjects (*P* < 0.01). However, no difference was observed in serum betatrophin levels between men and women. Stratification with BMI in the T2DM group or control subjects showed that serum concentrations of betatrophin were not changed in overweight/obese subjects (BMI ≥25 kg/m²) when compared with nonobese subjects (BMI <25 kg/m²) (Supplementary Tables 1 and 2).

Correlation of Betatrophin With Clinical Parameters

We next investigated the relationship of circulating betatrophin levels with various anthropometric parameters by using partial correlations. Serum betatrophin correlated positively with age, FPG, 2hPG, HbA_{1c}, FINS, and PSI but negatively with 1/HOMA-IR and ISI_M in the T2DM group (Table 2). All of these

Table 1—Clinical and biochemical characteristics in control subjects and in patients with T2DM

| Variables | Healthy control (n = 83) | T2DM (n = 83) | <i>P</i> value |
|--|--------------------------|------------------------|----------------|
| Age (years) | 47.96 ± 10.07 | 48.39 ± 10.97 | 0.797 |
| Sex, male/female (%) | 65.1 | 62.7 | 0.436 |
| BMI (kg/m ²) | 25.25 ± 3.63 | 25.12 ± 3.23 | 0.799 |
| WHR | 0.90 ± 0.06 | 0.92 ± 0.05 | 0.116 |
| Systolic blood pressure (mmHg) | 124 ± 13 | 128 ± 12 | 0.076 |
| Diastolic blood pressure (mmHg) | 79 ± 10 | 79 ± 8 | 0.876 |
| Alcohol use (%) | | | |
| Regular drinker | 7.2 | 10.8 | 0.443 |
| Occasional drinker | 19.3 | 24.1 | 0.286 |
| Never | 73.5 | 65.1 | 0.156 |
| Smoking status (%) | | | |
| Current smoker | 15.7 | 19.3 | 0.490 |
| Former smoker | 14.4 | 14.4 | 0.587 |
| Never | 69.9 | 66.3 | 0.370 |
| Glucose ₀ (mmol/L) | 5.06 ± 0.45 | 9.99 ± 3.05 | 0.000 |
| ALT* (U/L) | 21 (17–34) | 27 (16–46) | 0.321 |
| AST* (U/L) | 21 (18–26) | 22 (16–29) | 0.851 |
| GGT* (U/L) | 24 (14–41) | 34 (20–67) | 0.013 |
| Glucose ₃₀ (mmol/L) | 7.83 ± 1.66 | 14.79 ± 3.41 | 0.000 |
| Glucose ₁₂₀ (mmol/L) | 5.82 ± 1.19 | 19.25 ± 5.55 | 0.000 |
| HbA _{1c} (%) | 5.26 ± 0.29 | 9.23 ± 2.19 | 0.000 |
| HbA _{1c} (mmol/mol) | 34.00 ± 3.22 | 77.36 ± 23.91 | 0.000 |
| TC (mmol/L) | 4.61 ± 0.70 | 5.14 ± 1.31 | 0.001 |
| Triglycerides (mmol/L) | 1.59 ± 0.94 | 2.46 ± 1.73 | 0.000 |
| LDL cholesterol (mmol/L) | 2.86 ± 0.62 | 3.28 ± 1.13 | 0.004 |
| HDL cholesterol (mmol/L) | 1.27 ± 0.34 | 1.16 ± 0.30 | 0.024 |
| Insulin ₀ (μU/mL) | 7.16 ± 3.05 | 8.27 ± 3.91 | 0.043 |
| Insulin ₃₀ (μU/mL) | 39.80 ± 26.78 | 21.25 ± 35.11 | 0.010 |
| Insulin ₁₂₀ (μU/mL) | 26.29 ± 15.90 | 34.17 ± 20.01 | 0.006 |
| 1/HOMA-IR | 0.74 ± 0.31 | 0.37 ± 0.22 | 0.000 |
| ISI _M | 12.91 ± 2.72 | 9.04 ± 2.61 | 0.000 |
| HOMA-β | 98.62 ± 47.03 | 31.48 ± 20.68 | 0.000 |
| InsAUC ₃₀ /GluAUC ₃₀ | 21.66 ± 12.88 | 8.17 ± 12.34 | 0.000 |
| InsAUC ₁₂₀ /GluAUC ₁₂₀ | 26.92 ± 16.19 | 10.47 ± 11.37 | 0.000 |
| Betatrophin* (pg/mL) | 296.57 (196.53–509.46) | 613.08 (422.19–813.08) | 0.000 |

Data are means ± SD, %, and median (25th and 75th percentiles). *Log-transformed variable, values given are median (25th and 75th percentiles).

correlations remained statistically significant despite adjustments for age, sex, and BMI. After Bonferroni correction was applied, the statistically significant association still remained except for FPG, HbA_{1c}, and FINS (Table 2). Multivariate regression analyses showed that age ($\beta = 0.010$; *P* = 0.000), 2hPG ($\beta = 0.021$; *P* = 0.000), and PSI ($\beta = 0.005$; *P* = 0.001) were independently related factors influencing serum betatrophin levels ($R^2 = 0.374$; *P* = 0.000). In the control group, there was a correlation with age ($r = 0.217$; *P* = 0.049), but no correlation between betatrophin level and other clinical parameters.

CONCLUSIONS

In this study, we have demonstrated for the first time that serum betatrophin concentrations were significantly increased in newly diagnosed and untreated T2DM patients compared with the control subjects. Nevertheless, our findings did not accord with a recent study performed by Fenzl et al. (15) in which serum betatrophin was found to be not elevated in patients with T2DM. The disparities may be due to the difference in sample sizes, oral hypoglycemic agents, and different disease duration of T2DM. In that study, a total of 76 subjects were recruited, including 1) lean

Table 2—Partial correlations analysis of variables associated with circulating betatrophin levels in patients with T2DM

| | Betatrophin* | | | Betatrophin (age, sex, and BMI adjusted) | | |
|--|--------------|----------------|----------------------------|--|----------------|---------------------------|
| | <i>r</i> | <i>P</i> value | Bonferroni <i>P</i> value† | <i>r</i> | <i>P</i> value | Bonferroni <i>P</i> value |
| Age | 0.324 | 0.002 | 0.048 | | | |
| BMI | 0.163 | 0.140 | 1.0 | | | |
| WHR | 0.033 | 0.765 | 1.0 | 0.019 | 0.877 | 1.0 |
| Systolic blood pressure | 0.110 | 0.321 | 1.0 | 0.072 | 0.547 | 1.0 |
| Diastolic blood pressure | 0.092 | 0.409 | 1.0 | 0.027 | 0.822 | 1.0 |
| ALT* | 0.078 | 0.482 | 1.0 | 0.124 | 0.301 | 1.0 |
| AST* | 0.116 | 0.298 | 1.0 | 0.146 | 0.222 | 1.0 |
| GGT* | 0.072 | 0.520 | 1.0 | 0.048 | 0.689 | 1.0 |
| Glucose ₀ | 0.342 | 0.002 | 0.048 | 0.346 | 0.003 | 0.072 |
| Glucose ₃₀ | 0.029 | 0.804 | 1.0 | 0.074 | 0.535 | 1.0 |
| Glucose ₁₂₀ | 0.393 | 0.000 | 0.000 | 0.359 | 0.002 | 0.048 |
| HbA _{1c} | 0.291 | 0.008 | 0.192 | 0.304 | 0.009 | 0.216 |
| TC | 0.092 | 0.406 | 1.0 | 0.072 | 0.546 | 1.0 |
| Triglycerides | 0.010 | 0.925 | 1.0 | 0.037 | 0.755 | 1.0 |
| LDL cholesterol | 0.050 | 0.656 | 1.0 | 0.025 | 0.832 | 1.0 |
| HDL cholesterol | −0.011 | 0.919 | 1.0 | −0.033 | 0.786 | 1.0 |
| Insulin ₀ | 0.284 | 0.009 | 0.216 | 0.267 | 0.023 | 0.552 |
| Insulin ₃₀ | 0.117 | 0.319 | 1.0 | 0.054 | 0.653 | 1.0 |
| Insulin ₁₂₀ | 0.305 | 0.005 | 0.120 | 0.353 | 0.002 | 0.048 |
| 1/HOMA-IR | −0.368 | 0.001 | 0.024 | −0.355 | 0.002 | 0.048 |
| ISI _M | −0.359 | 0.001 | 0.024 | −0.354 | 0.002 | 0.048 |
| HOMA-β | 0.065 | 0.560 | 1.0 | 0.039 | 0.743 | 1.0 |
| InsAUC ₃₀ /GluAUC ₃₀ | 0.126 | 0.282 | 1.0 | 0.069 | 0.567 | 1.0 |
| InsAUC ₁₂₀ /GluAUC ₁₂₀ | 0.150 | 0.200 | 1.0 | 0.113 | 0.346 | 1.0 |

*Log-transformed variable. †Bonferroni corrected *P* value; correction is made for multiple testing 24 times.

(*n* = 20) and morbidly obese individuals (*n* = 19) and 2) nondiabetic (*n* = 19) and T2DM individuals (*n* = 18). In addition, all patients with T2DM received metformin as baseline therapy, and five had sulfonylurea. It is not clear whether oral hypoglycemic agents will affect serum betatrophin level or not. What is more, in that study, mean disease duration in the T2DM patients was 8.3 ± 1.0 years. It is possible that serum betatrophin level could be affected by disease durations of T2DM.

The mechanisms underlying increased betatrophin levels in newly diagnosed T2DM patients remain elusive. The liver and pancreas arise from a common multipotent population of endoderm cells and couple with the robust β -cell proliferation response to tissue-specific insulin resistance in the liver (16–19). Recently, Yi et al. (2) reported that hepatic expression of betatrophin was upregulated in animal models of insulin resistance in which β -cell replication is increased. In our study, serum betatrophin levels were negatively associated with hepatic insulin sensitivity (1/HOMA-IR).

Furthermore, serum betatrophin levels correlated positively with 2hPG and PSI. Therefore, we speculate that the increase in serum betatrophin in T2DM might be attributable to a defensive response, which may represent an ability to adapt to hepatic insulin resistance or increased blood glucose concentrations by increasing β -cell proliferation and insulin secretion.

In our study, we have demonstrated that age positively correlated with circulating betatrophin levels, which is consistent with the results obtained by Espes et al. (20) and Fenzl et al. (15). Despite there being a well-documented decline in β -cell replication rate with age in both mouse and humans (21), Saisho et al. (22), in an autopsy study of 167 nondiabetic individuals aged 20–102 years, found that the calculated β -cell mass remained constant, and although there was no significant change in β -cell size with aging, the mean β -cell nuclear diameter increased with age, and the proportion of apoptotic β -cells was unchanged by advanced age. Therefore, the observed increases in serum

betatrophin levels from aged versus young subjects in our study may represent a compensatory mechanism in response to aging.

So far, mounting evidence from recent animal-based studies suggests that betatrophin plays a key role in lipid metabolism. Mice lacking betatrophin had a 70% reduction in plasma triglyceride levels compared with littermate control subjects (23). Adenovirus-mediated hepatic overexpression of betatrophin increased plasma triglyceride levels more than five-fold (24). However, contrary to the results of animal studies, in the present human study, we did not find significant association between serum betatrophin and triglyceride. This result corresponds with the observations by Espes et al. (20) and Fenzl et al. (15). The reasons for the inconsistency of our clinical findings with the animal data may be attributed to the fact that mouse betatrophin is highly enriched in adipose tissues and liver, but human betatrophin is predominantly expressed in liver (24). Although the study performed by Fenzl et al. (15)

found that serum betatrophin significantly associated with TC, LDL cholesterol, and apolipoprotein B in patients with long duration of T2DM (16), we were unable to support that conclusion in this current study, which focused on patients with newly diagnosed and untreated T2DM. The reason for the discrepancy is unclear and probably because of the difference in patient populations. Future studies are needed to elucidate this point.

Limitations of our study also deserve comment. First, this study is limited by its cross-sectional design and provides no temporal interpretation of reported associations. Second, betatrophin was not a prespecified end point of recruited T2DM subjects in the study, and measurements of betatrophin were made on stored samples, although the samples were relatively fresh. Third, our analyses are based on single measurements of blood betatrophin, which may not reflect betatrophin levels over time. Serial changes in serum betatrophin need to be measured at different stages of T2DM besides newly diagnosed T2DM patients to further clarify the role of betatrophin in the pathogenesis of T2DM.

In summary, our results indicate for the first time that circulating betatrophin concentrations were significantly increased in patients with newly diagnosed T2DM and associated with glucose homeostasis and insulin sensitivity. Future studies are required to address the role of betatrophin in the pathogenesis of T2DM.

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